GENOTYPIC CHARACTERIZATION OF *ALCALIGENES XYLOSOXIDANS* SUBSP. *DENITRIFICANS* (*AXD* HC01) AND FOUR RELATED STRAINS

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ABSTRACT
In symbiont therapy, an insect's natural symbionts are genetically modified to prevent the transmission of a pathogen. This strategy is currently under investigation as a way to control the spread of Pierce's disease (PD) of grapevine. PD is caused by the bacterium *Xylella fastidiosa* (*Xf*), which is transmitted by the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*). The five GWSS symbionts used in this research were identified through biochemical testing as *Alcaligenes xylosoxidans denitrificans* (*Axd*) Hc01, *Axd*1, *Axd*2, *Axd*3, and *Axd*4. The genetic relatedness of these bacteria, as well as their relationships to other bacterial species was analyzed using two highly conserved prokaryotic genes, the 16S rDNA sequence and the gyrase B sequence. These sequences were used to construct phylogenetic trees using the neighbor-joining method. Analysis of the 16S tree indicated that all of these bacteria were closely related to members of the genus *Pseudomonas*. The phylogenetic trees that were constructed using the gyrase B gene also supported the conclusion that these bacteria are closely related to members of the genus *Pseudomonas*. Further testing using the 16S-23S intergenic spacer region one is currently underway.

INTRODUCTION
One new potential management strategy for Pierce's disease (PD) of grapevine is the use of symbiont therapy. Symbiont therapy exploits the interactions among a pathogen-transmitting organism, its bacterial symbionts, and the pathogenic organism itself (Beard 2002). First, a bacterial symbiont that occupies the same niche as the pathogen must be identified. These symbionts are genetically modified to produce a molecule that hinders the spread of the pathogen in question. The genetically modified bacteria are re-introduced into the vector so that they can reduce its ability to transmit the pathogen in question. For this approach to be successful, the bacterial symbiont must be easily cultured and manipulated *in vitro*, and the genetic modification cannot alter their value to the host organism or their ability to occupy their niche. In addition, the bacterial symbionts cannot be pathogenic to either their host or to non-target organisms before or after the genetic modification (Durvasula 2003). Symbiont therapy has been investigated as a way to control the spread of Chagas disease (Beard 2002; Durvasula 2003), murine colitis (Steidler 2000), and HIV (Chang 2003).

For symbiont therapy to be effective in limiting the spread of PD, a culturable symbiont that inhabits the pre-cibarium and cibarium of the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis)* is required, since these areas are colonized by *Xf*. Three bacterial species that meet these requirements are *Chryseomonas* spp, *Ralstonia* spp, and *Alcaligenes* spp (Bextine 2004). The *Alcaligenes* species were of particular interest because they were frequently isolated from wild GWSS (Kuzina 2004) and because they could also successfully colonize the xylem of various plants, including citrus (Araujo 2002; Bextine 2005). Five *Alcaligenes* species were isolated from the mouthparts of GWSS and identified as *Alcaligenes xylosoxidans* subspecies *denitrificans* (*Axd*) using standard morphological and biochemical tests. Four of these species were designated as *Axd*1, *Axd*2, *Axd*3, and *Axd*4. The other *Alcaligenes* species that was found in GWSS was designated as *Axd* Hc01 and selected for further study (Bextine 2004). However, the classification of *Axd* Hc01 remains unsettled.

OBJECTIVE
1. If *Axd* Hc01 is to be used as part of a symbiont therapy program, the issues surrounding its identity must be resolved. One way to help clarify its identity and relationship to other identified *Axd* strains is to construct phylogenetic trees based on the sequences of universally present, highly conserved prokaryotic genes (Laguerre 1994). The goal of this research is to help identify *Axd* Hc01 by placing it in phylogenetic trees based on 16S, gyrase B, and 16S-23S intergenic spacer region sequences.

RESULTS

The phylogenetic trees based on 16S sequences indicate that *Axd* Hc01 is most closely related to members of the genus *Pseudomonas* (Figure 1). The second 16S phylogenetic tree shown also indicates that *Axd*1 and *Axd*2 are more closely related to *Axd* Hc01 than *Axd*3 and *Axd*4 (Figure 2). In the two gyrase B trees (Figures 3 and 4), *Axd* Hc01 does not group with the other *Alcaligenes* species used in the study, but some of its relatives do. In agreement with the 16S studies, *Axd* Hc01 groups most closely with a member of the genus *Pseudomonas* in the gyrase B trees (Figure 3). However, in the second gyrase B tree (Figure 4), *Axd* Hc01 does not group with members of this genus. Instead, *Axd*4 does. In the most current 16S-23S ITS 1 trees, *Axd* Hc01 does not group with any of the organisms included in the study (Figure 5). Its relatives *Axd*2 and *Axd*4 do cluster together in the bottom 16S-23S ITS 1 tree, and *Axd*1 groups with *Alcaligenes faecalis* 16.7 (Figure 6). The 16S-23S ITS 1 trees do not yet include all of the organisms that the first two trees do, and this could be one reason for the noted discrepancies. Once the 16S-23S ITS 1 trees are completed, the relationships among these five species will be further investigated and clarified using DNA-DNA hybridization techniques.

CONCLUSIONS

From a preliminary analysis of these results, it can be concluded that *Axd* Hc01 and its relatives are related to members of the genus *Pseudomonas*. However, more work using the 16S-23S ITS region will be necessary to provide more information concerning the identity of *Axd* Hc01 at the species and subspecies level and to clarify its relationship to *Axd*1, *Axd*2, *Axd*3, and *Axd*4. The successful identification of the *Axd* Hc01 bacterium and its relatives will help contribute to a strategy based on symbiont therapy to control PD.

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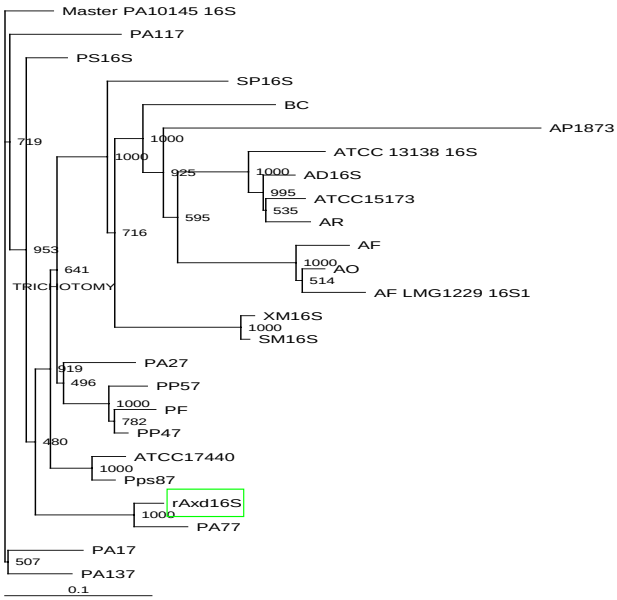
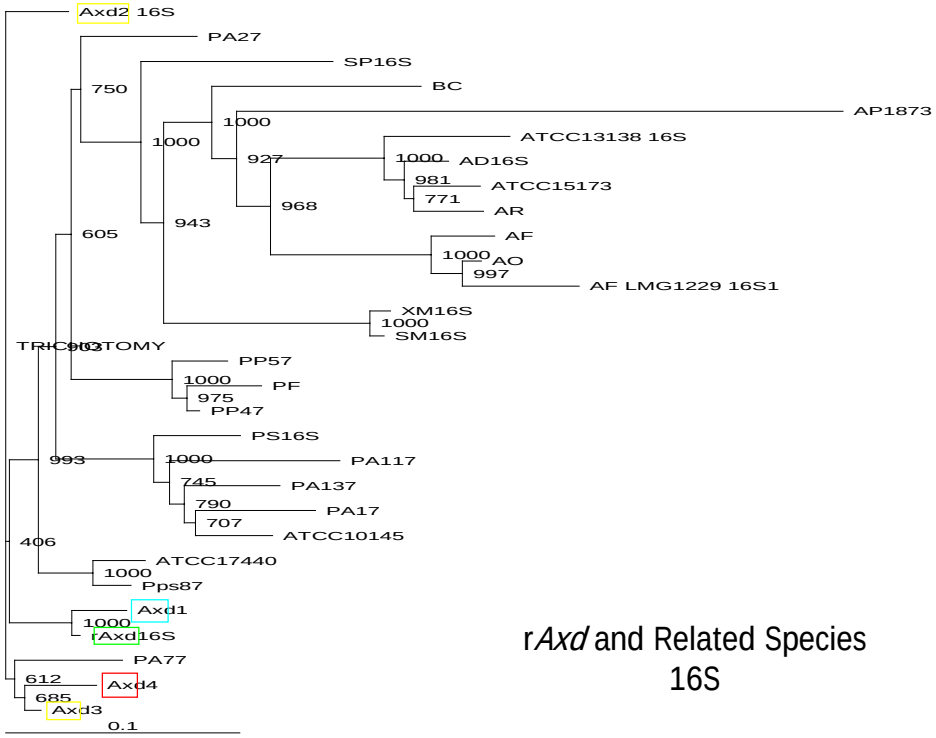


r*Axd* 16S

Figure 1. 16S phylogenetic tree inculding r*Axd*.



r*Axd* and Related Species 16S

Figure 2. 16S phylogenetic tree including r*Axd* and its relatives.

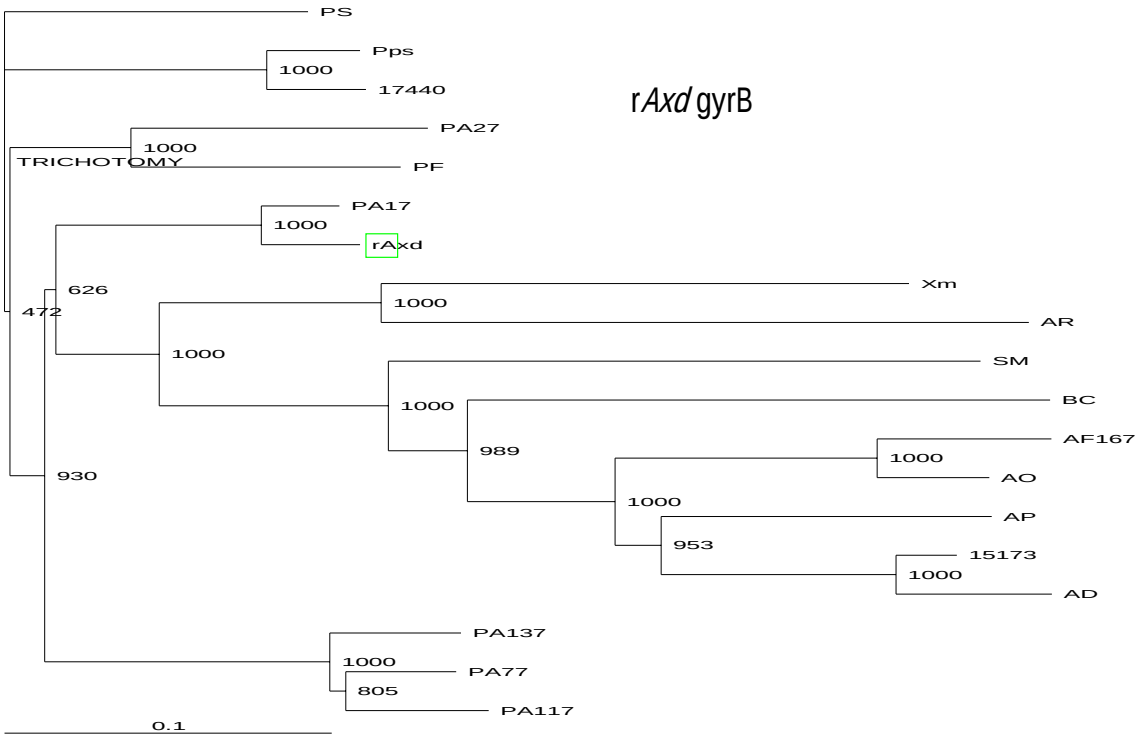


Figure 3. gyrB phylogenetic tree for r*Axd*.

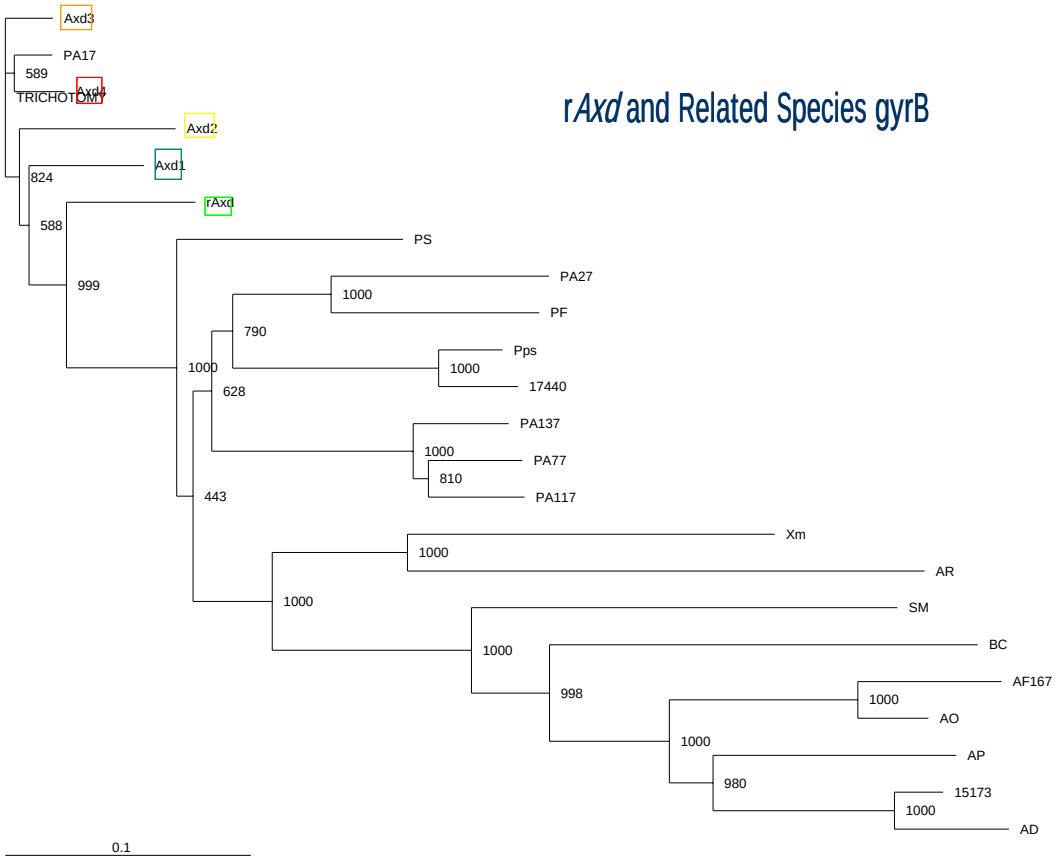


Figure 4. gyrB phylogenetic tree including r*Axd* and its relatives.

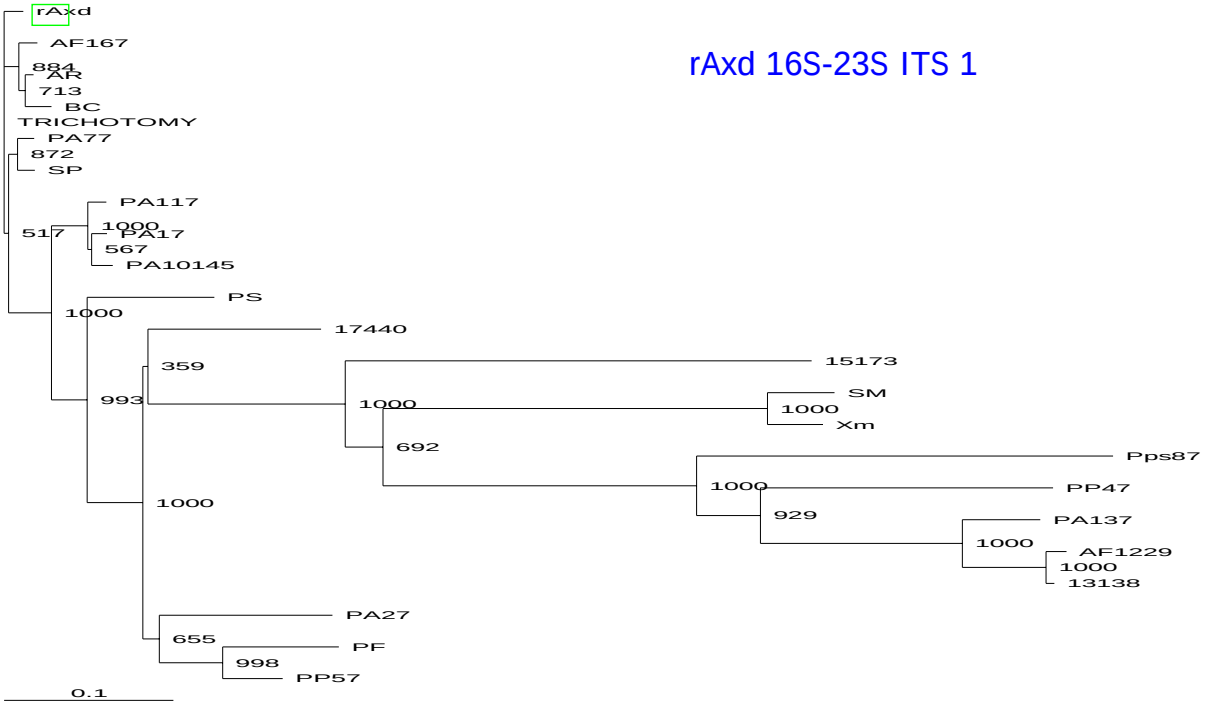


Figure 5. 16S-23S ITS 1 phylogenetic tree for r*Axd*.

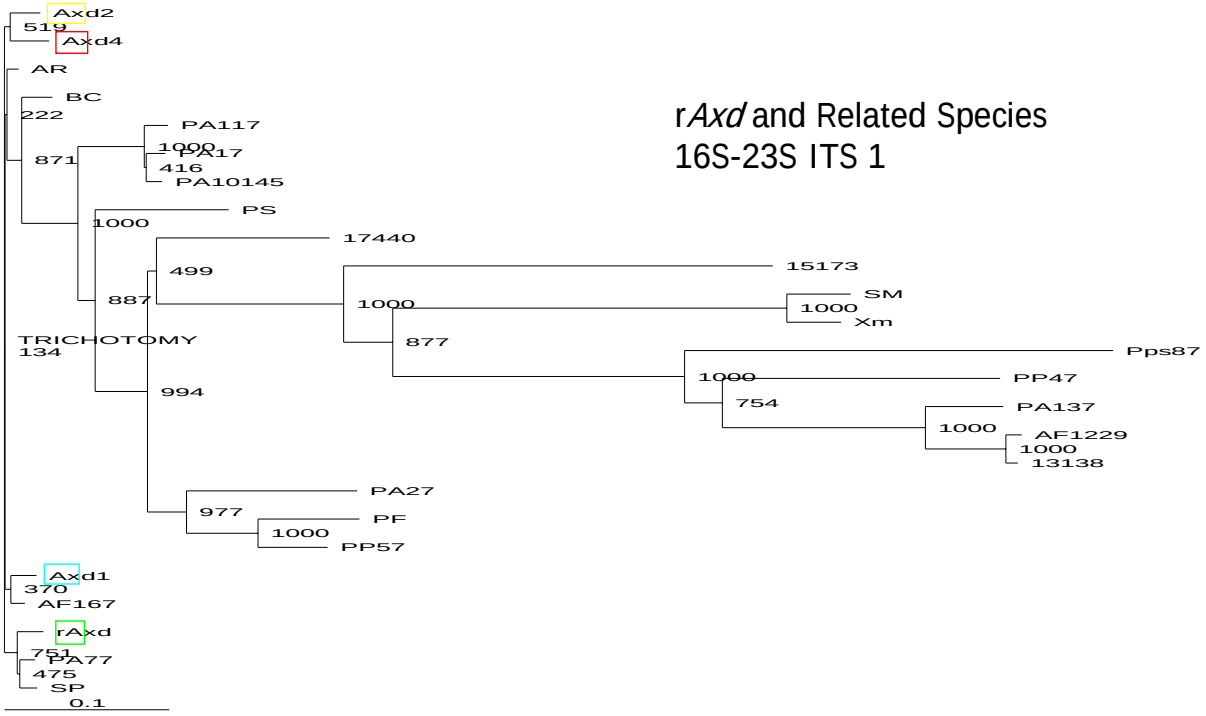


Figure 6. 16S-23S ITS 1 phyogenetic tree including r*Axd* and its relatives.

REGULATION OF BIOTECHNOLOGY APPLIED TO AGRICULTURE

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ABSTRACT
Regulatory agencies are faced with an increasing number of permit applications using biotechnology to solve agricultural problems. Newer innovations include engineered biopesticides, transgenic and paratransgenic insects such as the symbiotic control project aimed at controlling Pierce's disease. Review panels point to the regulatory burden as one reason not to fund new technology. The results of a Workshop held 7-9 November 2006 in Washington DC to address these and other regulatory issues will be described.